(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 093 855 A2

# (12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
**25.04.2001 Bulletin 2001/17**

(51) Int Cl.[7]: **B01L 3/02**

(21) Application number: **00309286.3**

(22) Date of filing: **20.10.2000**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE**
Designated Extension States:
**AL LT LV MK RO SI**

(30) Priority: **22.10.1999 JP 30162799**

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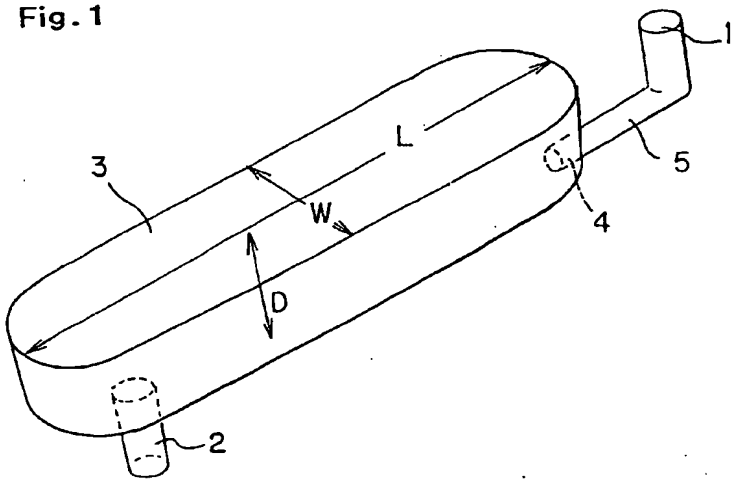
## (54) Micropipette and dispenser

(57) A micropipette includes: at least one substrate, an inlet port through which a sample is delivered from the outside, a cavity to be poured and filled with the sample, and an injection port for expelling the sample are formed on the at least one substrate. The substrate for forming the cavity is made of ceramics, a piezoelectric/electro-strictive element is provided for at least one wall surface of the substrate, and the sample moves as a laminar flow in the cavity. Volumes of the cavity are changed by driving the piezoelectric/electrostrictive element to expell a certain amount of the sample in the cavity from the injection port. According to the micropipette, it is possible to form microspots at a high accuracy and a high speed. According to a dispenser using the micropipette, it is possible to efficiently dispense hundreds to ten thousands of different samples at one time and form microspots. Therefore, the productivity is remarkably improved.

Fig. 1

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## Description

Background of the Invention and Related Art Statement

**[0001]** The present invention relates to a micropipette superior in droplet-volume controllability and productivity and preferably used to line and fix micro-volume droplets at a high density such as manufacturing of DNA chips and a dispenser using the micropipette.

**[0002]** Because the genetic-structure analyzing method has been recently remarkably advanced and many genetic structures including structures of human genes have been clarified. To analyze the above genetic structures, a DNA chip is used in which thousands to ten thousands or more of different types of DNA pieces are lined and fixed on a substrate such as a microscope slide glass as microspots.

**[0003]** As method for forming microspots in manufacturing the DNA chip, the QUILL method, pin-and-ring method, or spring pin method is widely used. Even when any method is used, it is necessary to decrease the fluctuation of volumes and shapes of microspots and keep the distance between microspots constant. Moreover, it is greatly expected that a new method superior in shape controllability and productivity of microspots is developed for further increase of density.

**[0004]** In this case, the QUILL method is a method for forming a microspot by storing samples in a concave portion formed at the tip of a pin, making the pin tip contact with a substrate, and thereby moving the samples in the concave portion onto the substrate. However, there is a problem on durability that the pin tip is deformed or damaged due to the contact with a substrate or a problem that cross contamination easily occurs due to imperfect cleaning of the samples stored in the concave portion.

**[0005]** The pin and ring method is a method for forming spots on a substrate by reserving a sample solution in a microplate with a ring and thereafter catching the sample in the ring with the tip of a pin so that the solution passes through the ring in which the solution is reserved. However, the number of types of samples that can be reserved at one time depends on the number of rings, which has been approx. several types so far. Therefore, to form microspots of thousands to ten thousands of types of samples, hundreds to thousands of cleaning and drying steps are also necessary. Thus, it is difficult to say that the productivity is always high.

**[0006]** Moreover, the spring pin method is a method for forming microspots by pressing a sample attached to the tip of a pin against a substrate and thereby, moving the sample onto the substrate, in which damage of the pin and the substrate is moderated by a double-pin structure having a built-in spring to spout the sample. However, because only one-time spotting can be basically performed by one-time reservation. Therefore, the method is inferior in productivity.

**[0007]** Furthermore, in case of these conventional microspot-forming methods, because every sample solution is carried onto a substrate while it is exposed to the atmosphere, troubles occur that the sample is dried while it is carried and spotting cannot be performed. Therefore, a problem occurs that a very expensive sample solution cannot be efficiently used.

**[0008]** Furthermore, a method for performing spotting by using the so-called ink-jet system practically used for a printer is studied. However, forming thousands to ten thousands of samples in separate channels has many problems from viewpoints of size and cost. Moreover, in case of the ink-jet system, it is necessary to previously fill a pump with samples without any bubbles before spotting. Therefore, much filling sample is necessary and therefore, the sample use efficiency is very inferior. Furthermore, in general, it is better for bubble discharge that a liquid moves through a channel including a pump chamber at a high speed and thereby, a sample is agitated in the channel and thus, when a delicate DNA solution is used as a sample, DNA may be damaged.

**[0009]** The present invention has been made to solve the above problems, and its object is to provide a micropipette making it possible to form microspots at a high accuracy and a high speed and a dispenser superior in productivity using the micropipette and capable of forming microspots by efficiently dispensing hundreds to ten thousands of different samples at one time.

Summary of the Invention

**[0010]** That is, the present invention provides a micropipette comprising:

at least one substrate,
an inlet port through which a sample is delivered from the outside, formed on said at least one substrate,
a cavity into which the sample is poured and which is filled with the sample, and
an injection port for expelling the sample are formed on said at least one substrate,
the substrate for forming the cavity being made of ceramics, at least one wall face of the substrate being provided with a piezoelectric/electrostrictive element, and the sample moving as a laminar flow in the cavity,

wherein volumes of the cavity are changed by driving the piezoelectric/electrostrictive element and a certain amount of the sample in the cavity is expelled from the injection port.

**[0011]** Because a micropipette of the present invention uses the above structure, a very small amount of a liquid is expelled through an injection port correspondingly to each time of driving of a piezoelectric/electrostrictive element and the volume of the liquid is very small and constant. The driving cycle can correspond to a high frequency by using the piezoelectric/electrostrictive el-

ement and the time required for injection is also decreased. Moreover, because a sample moves in a closed space during the period until the sample is expelled after it is delivered, the sample is not dried during the period. Furthermore, because the substrate can be compactly formed, it is possible to shorten a channel through which a sample moves and reduce the deterioration of use efficiency due to attachment of the sample to the channel wall.

**[0012]** In case of a micropipette of the present invention, it is preferable to previously fill a cavity with a displacement liquid such as a buffer solution or physiologic saline solution, then deliver a sample into the cavity through the inlet port while laminar-flow-replacing a displacement liquid with the sample, and thereafter expel the sample in the cavity through an injection port by driving a piezoelectric/electro-strictive element. It is possible to control the terminal of completion of a laminar flow-replacing with a replacement time by previously obtaining the moving speed and the volume of the sample. However, it is more preferable to grasp the terminal by detecting the change of fluid characteristics in the cavity. Moreover, it is permitted to laminar-flow-replace a displacement liquid with the sample into the cavity from the inlet port while driving the piezoelectric/electrostrictive element. By previously securely replacing the inside of a cavity with an inexpensive replacement solution and then laminar-flow-replacing an inexpensive solution with an expensive sample, it is possible to completely prevent miss-injection from occurring and efficiently expel the expensive sample.

**[0013]** Moreover, in case of a micropipette of the present invention, it is preferable to previously fill a cavity with a replacement solution such as a buffer solution or physiologic saline solution, then deliver a sample into the cavity through the inlet port while replacing a replacement solution with the sample, grasp the terminal of completion of replacement by detecting the change of fluid characteristics in the cavity, and thereafter expel the sample in the cavity through an injection port by driving a piezoelectric/electrostrictive element. By detecting the change of fluid characteristics in the cavity and thereby grasping the completion of replacement, it is possible to easily distinguish between a portion where a sample mixes with a replacement solution and a portion where they do not mix each other and accurately clarify the portions even if they slightly mix in a channel. Therefore, it is possible to decrease the quantity of the sample mixed with the replacement solution that must be purged and improve the use efficiency of the sample.

**[0014]** Moreover, it is preferable to grasp the change of fluid characteristics in the cavity by applying a voltage for exciting vibrations to the piezoelectric/electrostrictive element and detecting the change of electric constants due to the vibrations. Thus, it is unnecessary to set a special detection element and inexpensive and high-accuracy detection is realized.

**[0015]** In case of a micropipette of the present invention, it is preferable that a sample inlet port, cavity, a sample injection port, and piezoelectric/electrostrictive element are formed at a plurality of places in one substrate or a plurality of units in each of which a sample inlet port, a cavity, a sample injection port, and the piezoelectric/electrostrictive element are formed in the above substrate are fixed to a fixing jig, moreover three types of portions such as a combination of a cavity and a piezoelectric/electrostrictive element, a sample inlet port, and a sample injection port are separately formed on at least two types of substrates and joined each other, and furthermore, at least a cavity and a piezoelectric/electrostrictive element are formed in/on the above one substrate, a unit formed by joining the above one substrate or more to one substrate on which one of either of a sample inlet port and a sample injection port or more are formed is formed and the one unit or more are fixed and integrated.

**[0016]** Because each portion is formed at a plurality of places in one substrate, it is possible to compactly arrange the portions, form injection ports at a high accuracy and a high density, and expel a plurality of types of samples at the same time. By fixing a plurality of units in each of which one portion is formed in one substrate to constitute the whole, each substrate is easily manufactured and the yield is improved. Moreover, by joining at least two substrates on each of which portions are formed as the whole, the range for selecting materials of the substrate is widened and it is possible to select an optimum material for each portion. Moreover, the yield of elements can be improved, the accuracy of an injection port can be improved, injection ports can be arranged at a high density, and a plurality of types of samples can be expelled at the same time.

**[0017]** Furthermore, it is preferable that a substrate is flat and injection ports of samples are formed on a side face or a major surface of the substrate, or that a substrate is flat, injection ports of samples are formed on one of opposite major surface of the substrate, and inlet ports of samples are formed on the other major surface of the substrate. By forming a substrate to be flat, the substrate can be manufactured by laminating green sheet described later and the whole becomes thin and compact. When injection ports are formed on a major surface of a substrate, it is possible to set the substrate in parallel with a flat plate on which injection ports are formed and easily keep the injection distance of droplets constant, and shapes of droplets are stabilized. Moreover, when injection ports are formed on the side face of a substrate, it is possible to longitudinally arrange flat substrates and thereby easily increase the density of the injection ports. Furthermore, by forming an inlet port and an injection port on opposite major surfaces, the length of a channel extending from the inlet port up to the injection port requires almost only the thickness of a flat plate, the channel of a sample solution is shortened and becomes simple, the frequency of a trouble that bubbles are caught in the channel to cause miss-injection can

be decreased, and moreover the sample use efficiency is improved.

**[0018]** Furthermore, it is permitted that two or more sample inlet ports are connected to one cavity. In case of this structure, it is possible to securely fill the cavity with samples by sucking or ejecting samples or a replacement solution through a plurality of inlet ports by adjusting the timing.

**[0019]** Furthermore, in case of a micropipette of the present invention, it is preferable that a substrate in/on which a cavity and a piezoelectric/electrostrictive element are formed is made of zirconium ceramics or every substrate is made of zirconium ceramics. It is preferable that these substrates are manufactured by the green-sheet laminating and sintering method. Zirconia, particularly stabilized zirconia and partially stabilized zirconia are suitable because they have a large mechanical strength, a high toughness, a large durability to an acid/alkaline solution, and a small reactivity with a piezoelectric film or electrode material. Moreover, it is permitted that a substrate on which at least one inlet port and one injection port are formed is made of a metal or resin superior in formability and cost.

**[0020]** Furthermore, a piezoelectric/electrostrictive film used for a piezoelectric/electrostrictive element is preferable because it is mainly made of lead zirconate, lead titanate, and lead magnesium niobate and thereby, it has a high electromechanical coupling factor and a high piezoelectric constant, a small reactivity with a substrate (zirconia ceramics) when a piezoelectric film is sintered, and a stable composition.

**[0021]** Furthermore, the present invention provides a dispenser using a plurality of micropipettes respectively formed so that inlet ports through which a sample is delivered from the outside, cavities to be filled with the sample, and injection ports for expelling the sample are formed on at least one substrate, a piezoelectric/electrostrictive element is provided for at least one wall surface of the substrate for forming the cavities, and the sample moves as a laminar flow in the cavity, wherein the injection ports are vertically and horizontally lined and arranged and different types of solution samples are injected from the injection ports.

**[0022]** Furthermore, the present invention provides a dispenser using a plurality of micropipettes respectively formed so that inlet ports through which a sample is delivered from the outside, a cavity into which the sample is poured and which is be filled with the sample, and injection ports for expelling the sample are formed on at least one substrate, the substrate forming the cavity is made of ceramics, the substrate has a piezoelectric/electrostrictive element on at least one wall surface, the cavity is previously filled with a displacement solution, then the sample is poured into the cavity through the inlet ports while replacing a displacement solution with the sample, completion of sample replacement in the cavity is grasped by detecting the change of fluid characteristics in the cavity, and thereafter a volume of the cavity is changed by driving the piezoelectric/electrostrictive element and a certain amount of the sample in the cavity is expelled through the injection ports, wherein the injection ports are vertically and horizontally lined and arranged and different types of solution samples are expelled from the injection ports.

**[0023]** These dispensers make it possible to supply many types of samples at the same time by using a plurality of micropipettes and easily replace a locally-defective pipette with a new one. Moreover, because injection ports are vertically and horizontally lined and arranged, each of the above dispensers is preferably adopted when two-dimensionally lined and fixed microspots like a DNA chip are necessary.

**[0024]** It is preferable that each of these dispensers is provided with a mechanism in which cartridges separately filled with different types of solution samples are set to sample inlet ports to deliver different solution samples through inlet ports in order to improve the sample use efficiency and moreover, provided with a mechanism in which a cartridge filled with a water-soluble solvent or organic solvent is set to each sample inlet port to clean the space from inlet ports up to injection ports formed in the substrate in order to expel thousands to ten thousands of DNA pieces to very small spots without contamination and at a high purity.

**[0025]** Moreover, it is preferable that each of the dispensers has a different-directional-flying-droplet shielding plate made of a thin plate having a hole coaxial with an injection port outside the injection port. Thus, even if the expelling direction of an injection droplet is deviated, the droplet does not reach a substrate. Therefore, it is possible to prevent a trouble that a spotting position is shifted or a spot mixes with a next spot.

## Brief Description of the Drawings

**[0026]** Fig. 1 is an illustration showing an example of a cavity.

**[0027]** Fig. 2 is a sectional view showing a micropipette of the present invention.

**[0028]** Figs. 3(a) and 3(b) show another type of a micropipette of the present invention. Fig. 3(a) is a top view and Fig. 3(b) is an A-A sectional view of Fig. 3(a).

**[0029]** Figs. 4(a), 4(b), 4(c), and 4(d) show still another type of a micropipette of the present invention. Fig. 4 (a) is a top view, Fig. 4(b) is a side view, Fig. 4(c) is a top enlarged view of each unit, and Fig. 4(d) is a sectional view of Fig. 4(c).

**[0030]** Figs. 5(a) and 5(b) show still another type of a micropipette of the present invention. Fig. 5(a) is a top view and Fig. 5(b) is a B-B sectional view of Fig. 5(a).

**[0031]** Figs. 6(a) and 6(b) show still another type of a micropipette of the present invention. Fig. 6(a) is a top view and Fig. 6(b) is a C-C sectional view of Fig. 6(a).

**[0032]** Figs. 7(a) and 7(b) show still another type of a micropipette of the present invention. Fig. 7(a) is a top view and Fig. 7(b) is a D-D sectional view of Fig. 7(a).

**[0033]** Figs. 8(a) and 8(b) show still another type of a micropipette of the present invention. Fig. 8(a) is a top view and Fig. 8(b) is an E-E sectional view of Fig. 8(a).

**[0034]** Figs. 9(a) and 9(b) show still another type of a micropipette of the present invention. Fig. 9(a) is a top view and Fig. 9(b) is an F-F sectional view of Fig. 9(a).

**[0035]** Fig. 10 is a perspective view showing a dispenser.

**[0036]** Figs. 11(a) and 11(b) show the micropipette used for the dispenser in Fig. 10. Fig. 11(a) is a top view and Fig. 11(b) is a G-G sectional view of Fig. 11(a).

**[0037]** Fig. 12 is a perspective view showing a state of setting a cartridge to a dispenser.

Detailed Description of Preferred Embodiment

**[0038]** In case of the basic structure of a micropipette of the present invention, a sample inlet port, a cavity to be filled with a sample, and a sample injection port are formed on at least one substrate and a piezoelectric element is provided for at least one wall surface forming the cavity of the substrate. Moreover, the micropipette preferably has a structure that the sample moves as a laminar flow in the cavity. The micropipette having such a structure is able to efficiently form a microspot such as a DNA chip at a high accuracy and a high speed by changing volumes in a cavity in accordance with the driving of a piezoelectric/electrostrictive element and expelling a certain amount of a sample in the cavity through an injection port.

**[0039]** The present invention will be described below in detail in accordance with the embodiments shown in the accompanying drawings. However, the present invention is not restricted to the embodiments.

**[0040]** Fig. 2 shows a micropipette of the present invention.

**[0041]** In Fig. 2, a nozzle portion 11 is formed by forming a thin-wall flat nozzle plate 13 provided with an injection port 12 having at least one nozzle hole with a zirconia-ceramics green sheet, and a pump portion 21 is formed by forming a spacer plate 25 on which at least one chamber portion 28 is formed and a blocking plate 23 laminated on one side of the spacer plate 25 to cover the chamber portion 28 with a zirconia-ceramics green sheet respectively, and the whole is laminated and integrally sintered to constitute a substrate 10. Moreover, the blocking plate 23 is provided with a sample inlet port 16 and connected to an introduction hole 14 and a communication path 17 connected with the chamber portion 28 formed on the spacer plate 25.

**[0042]** Furthermore, a piezoelectric/electrostrictive element 22 having a lower electrode 31, a piezoelectric/electrostrictive layer 32, and an upper electrode 33 are formed on the outside face of the blocking plate 23.

**[0043]** According to the micropipette having the above structure, it is possible to manufacture a DNA chip lined and fixed as a microspot on a substrate such as microscope slide glass because, when an electric field is generated between the upper electrode 33 and the lower electrode 31, the piezoelectric/electrostrictive layer 32 is deformed, the volume of a cavity (pressuring chamber) 15 formed because the chamber portion 28 is covered is decreased, and thereby a sample (solution containing DNA fragment) filling the cavity 15 is expelled from the injection port 12 communicating with the cavity 15 at a predetermined speed. Moreover, as shown in Fig. 2, the structure of the so-called ink-jet system is disclosed in, for example, the specification of Japanese Patent Laid-Open No. 40030/1994 and therefore, it is possible to refer to the specification.

**[0044]** In case of the micropipette having the above structure, a shape and dimensions of passage is formed to move solution sample containing DNA fragment as a laminar flow in the cavity (pressuring chamber) 15.

**[0045]** A specific cavity will be described below by referring to Fig. 1. A cavity 3 is slender as shown in Fig. 1 and has a shape in which an inlet port 1 or an introduction port 4 for introducing a sample is formed at one end of the cavity 3 and an injection port 2 is connected to the other end of it. By forming the cavity 3 into the above shape, a sample moving into the cavity 3 from the inlet port 1 or through a communication path 5 and an introduction port 4 from the inlet port 1 can be led to the injection port 2 without disturbing the flow of the sample by using the cavity 3 as a part of the passage extending from the inlet port 1 up to the injection port 2. Specific dimensions of the cavity 3 depend on the type of a sample, the size of a droplet to be formed, or the droplet forming density. For example, in case of a micropipette for manufacturing a DNA chip requiring hundreds-of-micron-diameter droplet spotting of a sample obtained by dispersing sample liquid containing DAN fragment having 1 bp to 10,000 bp in a ×3SSC buffer solution {0.45M-sodium-chloride 0.045M-sodium-citrate aqueous solution (pH 7.0)} at a concentration of 1 μg/μl at hundreds-of-micron pitch, it is preferable to set a cavity length (L) to 1 to 5 mm, a cavity width (W) to 0.1 to 1 mm, and a cavity depth (D) to 0.1 to 0.5 mm. Moreover, it is preferable that the inner wall of the cavity is smooth so that there is no protrusion that disturbs a flow and the cavity is made of ceramics having a high affinity for a sample solution.

**[0046]** Moreover, in case of a micropipette of the present invention, it is preferable to previously fill a cavity with a buffer solution or physiological saline solution and then, pour a sample into the cavity through an inlet port while laminar-flow-replacing with the sample, and thereafter drive a piezoelectric/electrostrictive element. Moreover, in this case, it is preferable to grasp the completion of laminar flow replacement of the sample in the cavity by detecting the change of fluid characteristics in the cavity. Furthermore, it is preferable that the replacement of the sample with a displacement liquid is carried out in the form of a laminar flow. However, when characteristics of samples are changed or the solution moving speed is very high, or in case of the inside of the

cavity nearby an introduction hole, it is not always necessary to use a laminar flow. In this case, though the amount of the sample to be purged increases due to mixing of the sample with the displacement liquid, it is possible to minimize the increase of the amount of the sample to be purged by detecting the change of fluid characteristics in the cavity and thereby judging the completion of replacement. In this case, the change of fluid characteristics in the cavity is grasped by applying a voltage for exciting vibrations to the piezoelectric/electrostrictive element and detecting the change of electric constants due to the vibrations. The above detection of the change of fluid characteristics is disclosed in the specification of, for example, Japanese Patent Laid-Open No.201265/1996 and therefore, it is possible to refer to the contents of the specification.

**[0047]** Specifically, electrical connection from a driving power supply is disconnected from an optional piezoelectric/electro-strictive element by a relay at a predetermined interval and simultaneously, means for measuring a resonance frequency is connected by the relay to electrically measure the impedance or resonation frequency at that point of time. Thereby, it is possible to grasp whether the viscosity and specific gravity of a solution is equal to those of a purposed sample (solution containing DNA fragment and the like). That is, in the case of a micropipette of the present invention, it is possible to simplify the structure of the micropipette because the micropipette functions as a sensor.

**[0048]** Then, a micropipette of the present invention is able to pour a displacement liquid such as a buffer solution or physiological saline solution through an inlet port into a cavity while expelling a sample, similarly, completely discharge the sample remaining in the cavity through laminar-flow replacement, and prepare for the next sample injection. In this case, detecting whether a sample remains in a cavity (whether the sample can be expelled as a sample) can be also grasped by detecting the change of fluid characteristics in the cavity. Thus, by using a micropipette of the present invention, it is possible to greatly decrease the amount of unused sample to be wasted through laminar-flow replacement or by a replacement-completion detecting mechanism and improve the sample use efficiency.

**[0049]** Figs. 3(a) and 3(b) to Figs. 9(a) and 9(b) show other types of micropipettes of the present invention.

**[0050]** In Figs. 3(a) and 3(b), pluralities of sample inlet ports 16, cavities 15, sample injection ports 12, and piezoelectric/electrostrictive elements 22 are formed in/on one substrate 40 and the upper electrode 33 and lower electrode 31 of each piezoelectric/electrostrictive element 22 are collectively extended outward. This is preferable because it is possible to expel different types of samples at the same time and efficiently manufacture DNA chips at a high productivity.

**[0051]** The micropipette in Figs. 4(a) and 4(b) shows an embodiment formed by fixing a plurality of units in each of which one sample inlet port 16, one cavity 15, sample injection port 12, and one piezoelectric/electrostrictive element 22 are formed in/on one substrate {see Figs. 4(c) and 4(d)} to a fixing jig 35 (general name of a pressing jig 18, positioning pin 19, and fixing plate 20). Each unit is fixed to the fixing plate 20 by the positioning pin 19 and the pressing jig 18 for holding a tube 17 (communication path) for supplying a sample to the sample inlet port 16. Though each unit is fixed by fastening both ends of the pressing jig 18 to the fixing plate 20 by a screw 35A in Figs. 4(a) and 4(b), it is also possible to mechanically fix each unit by a screw and a spring or fix each unit by an adhesive.

**[0052]** A substrate 40 on which the sample inlet port 16, cavity 15, and sample injection port 12 are formed shown in Figs. 3(a) and 3(b) and Figs. 4(a) to 4(d) is made of ceramics and moreover, it can use one of stabilized zirconia, partially stabilized zirconia, alumina, magnesia, and silicon nitride. Among these materials, stabilized or partially stabilized zirconia is most preferably used because it has a large mechanical strength, a high toughness, and a small reactivity with a piezoelectric film or electrode material even in the form of a thin plate. Moreover, when stabilizd or partially stabilized zirconia is used as the material of the substrate 40 or the like, it is preferable that a portion on which the piezoelectric/electrostrictive element 22 is formed contains an additive such as alumina or titania. Moreover, the piezoelectric/electrostrictive layer of the piezoelectric/electrostrictive element 22 can use composite ceramics containing the component of one of lead zirconate, lead titanate, lead magnesium niobate, lead magnesium tantalate, lead nickel niobate, lead zinc niobate, lead manganese niobate, lead antimony stannate, lead manganese tungstate, lead cobalt niobate, and barium titanate or a combination of any of the above substances. In case of the present invention, a material mainly containing the component consisting of lead zirconate, lead titanate, and lead magnesium niobate is preferably used. This is because the above material has not only a high electromechanical coupling factor and a high piezoelectric constant but also a small reactivity with a substrate material when a piezoelectric film is sintered and thereby, makes it possible to stably form an object having a predetermined composition.

**[0053]** Moreover, it is permitted to use the ceramics which contain the oxides or the like of the following substances as an independent substance or a mixture in addition to the above piezoelectric ceramics: lanthanum, calcium, strontium, molybdenum, tungsten, barium, niobium, zinc, nickel, manganese, cerium, cadmium, chromium, cobalt, antimony, iron, yttrium, tantalum, lithium, bismuth, and tin or the like. For example, it is preferable to use ceramics mainly consisting of lead zirconate, lead titanate, and lead magnesium niobate and moreover, containing lanthanum and/or strontium.

**[0054]** It is preferable that upper electrode and lower electrode of a piezoelectric/electrostrictive element is solid at room temperature and consists of a conductive

metal. For example, it is permitted to use one of metals alone such as aluminum, titanium, chromium, iron, cobalt, nickel, copper, zinc, niobium, molybdenum, ruthenium, palladium, rhodium, silver, tin, tantalum, tungsten, iridium, platinum, gold, and lead, or an alloy obtained by combining any ones of these metals and moreover, use cermet obtained by dispersing a material same as that of a piezoelectric film in the above metals. A substrate, piezoelectric/electrostrictive element, and electrode made of any one of the above materials are used for all embodiments of the present invention in common.

**[0055]** Figs. 5(a) and 5(b) show an embodiment of a micropipette composed of a substrate 40 having a cavity 15, a piezoelectric/electrostrictive element 22, and an introduction hole 14, a substrate 39 having a set of one inlet port 16 and two communication paths 17, and a substrate 38 having a plurality of injection ports 12, the substrates 40, 39, and 38 being joined into one body by an adhesive 34. The substrate 40 is made of partially stabilized zirconia, the substrate 39 is made of stainless steel, and the substrate 38 is made of polyimide resin. Though it is permitted to mechanically join the substrates each other, it is preferable to join them by an adhesive or through thermal diffusion in order to keep the channel sealing characteristic.

**[0056]** An adhesive to be used is properly selected from the viewpoints of the combination of substrate material and thermal expansion coefficient and stability against sample-solution. It is suitable to use one of vinyl-, acrylic-, phenol-, polyamide-, resorcinol-, urea-, melanin-, polyester-, epoxy-, furan-, polyurethane-, silicon-, rubber-, polyimide-, and polyolefin-based adhesives. Particularly, epoxy-and polyimide-based adhesives are preferable from the viewpoints of adhesive strength and durability. Moreover, it is possible to use each adhesive mixed with very small beads made of glass or the like in order to keep the thickness of the adhesive constant.

**[0057]** Figs. 6(a) and 6(b) show another embodiment of a micropipette of the present invention. This micropipette is referred to as the so-called edge type, in which a sample inlet port 16, a cavity 15, a sample injection port 12, and a piezoelectric/electrostrictive element 22 are formed at a plurality of places in/on one substrate 40. Moreover, in the case of this micropipette, the sample injection port 12 is formed on the side face of the substrate 40 and a sample delivered into the sample inlet port 16 from a normal micropipette 45 passes through a communication path 17 in the substrate 40, and enters and fills the cavity 15. The micropipette changes volumes in the cavity 15 by driving the piezoelectric/electrostrictive element 22 to expel a certain amount of the sample filling the cavity 15 through the injection port 12.

**[0058]** Figs. 7(a) and 7(b) show still another embodiment of a micropipette of the present invention. This micropipette is referred to as the so-called face type same as those shown in Figs. 3(a) and 3(b) to Figs. 5(a) and 5(b), in which a sample inlet port 16, a cavity 15, a sample injection port 12, and a piezoelectric/electro-strictive element 22 are formed at a plurality of places in/on one substrate 40 similarly to the case of Figs. 6(a) and 6(b). Moreover, in case of this micropipette, the sample injection port 12 is formed on a major surface of the substrate 40. The cavity 15 and the sample inlet port 16 are connected by an introduction hole 14 and a communication path 17.

**[0059]** Figs. 8(a) and 8(b) show an embodiment in which a substrate 40 is formed into a flat plate, a sample injection port 12 is formed on one of opposite major surface of the substrate, and a sample inlet port 16 is formed on the other major surface of the substrate. The piezoelectric/electrostrictive element 22 is formed on the major surface same as the injection port.

**[0060]** Figs. 9(a) and 9(b) show an embodiment in which two sample inlet ports 16 are connected to one cavity 15. A piezoelectric/electrostrictive element 22 is formed on the same major surface as the sample inlet ports 16 and a sample injection port 12 is formed on the other major surface.

**[0061]** Then, a dispenser using one of the above micropipettes will be described. Fig. 10 shows a dispenser 55.

**[0062]** The dispenser 55 in Fig. 10 is formed by vertically setting a plurality of micropipettes 50 (50a, 50b, and 50c) respectively having the sample inlet port 52 and sample injection port 51 shown in Figs. 11(a) and 11(b) while turning the sample injection ports downward. That is, the micropipettes 50a, 50b, and 50c are formed so that sample inlet ports 52a, 52b, and 52c are turned upward, sample injection ports 51a, 51b, and 51c are turned downward and vertically and horizontally lined and arranged and different types of solution samples are expelled through the sample injection ports 51a, 51b, and 51c. A different-directional-flying shielding plate 53 made of a thin plate having a hole coaxial with an injection port is set further below the sample injection ports 51a, 51b, and 51c.

**[0063]** It is preferable that the dispenser 55 having the above structure is provided with a mechanism in which a cartridge 60 whose holes are filled with different types of solution samples is set to the sample inlet ports 52a, 52b, and 52c one to one to expel different solution samples through the discharge ports 51a, 51b, and 51c as shown in Fig. 12 because samples can be efficiently expelled. Moreover, it is preferable that the dispenser 55 is provided with a mechanism in which a cartridge filled with a physiological saline solution or organic solvent is set to each sample inlet port to clean the space expanding from inlet ports up to injection ports formed in a substrate in order to expel thousands to ten thousands of DNA fragments to very small spots without contamination and at a high purity. To deliver a sample or the like into each sample inlet port from a cartridge, it is also permitted to use a method of setting a cartridge to an inlet port and then opening the bottom of the cartridge with a needle or the like or a method of previously form-

ing a needle or the like nearby an inlet port so that a cartridge is opened at the same time when setting the cartridge. Moreover, it is permitted to add a mechanism for forcibly feeding gas or the like after opening a cartridge and forcibly pushing out a sample or the like.

**[0064]** Then, a DNA-chip manufacturing method using the dispenser 55 of the present invention will be described below.

**[0065]** In general, a sample containing DNA fragments to be spotted for a DNA chip is used by amplifying the DNA fragments in the cartridge 60 shown in Fig. 12. However, in case of a dispenser of the present invention using a micropipette having a slight space in a substrate, it is permitted to perform amplification in the micropipette.

**[0066]** When the DNA fragments are amplified in the cartridge 60, the cartridge filled with a buffer solution serving as displacement liquid is previously set and then, the cavity of each micropipette is filled with the buffer solution, and moreover the cartridge filled with a DNA-fragment sample is set to an inlet port, and the bottom of the cartridge is opened by a needle or the like to deliver the sample into the inlet port. Thereafter, the cavity is laminar-flow-replaced with the sample while expelling the previously-poured buffer solution through the injection port by driving a piezoelectric/electrostrictive element.

**[0067]** A replacement completion point is detected by making the piezoelectric/electrostrictive element function as a sensor for detecting the viscosity and specific gravity of the solution in the cavity by switching a relay. After replacement is completed, a DNA chip is manufactured by driving the piezoelectric/electrostrictive element in accordance with an element driving condition suitable for the number of droplets corresponding to a required spot diameter and repeating spotting. In general, one spot is formed by expelling one droplet to hundreds of droplets from a micropipette. When the amount of the sample in an inlet port is decreased, it is possible to completely use the sample without leaving the sample in the micropipette by adding a buffer solution and continuing expelling so that bubbles do not enter a channel. Completion of replacement of the sample with a displacement liquid (completion of sample expelling) is performed by similarly detecting the viscosity and specific gravity of the solution with the piezoelectric/electrostrictive element. Moreover, it is preferable to use a method by using a sample solution whose concentration is previously lowered, and drying a solvent while forming microspots on a substrate. By using this method, it is possible to further reduce the amount of a sample remaining in a channel and improve the sample use efficiency.

**[0068]** Furthermore, it is preferable to use a displacement liquid and sample from which a dissolved gas is previously removed through deaeration. By using such a deaerated solution, it is possible to avoid a trouble that bubbles are caught in a channel and thereby the channel cannot be filled with a solution because bubbles are dissolved in the solution when filling the channel with the solution and prevent an expelling trouble that bubbles are produced in a fluid while the fluid is expelled.

**[0069]** As described above, a micropipette of the present invention makes it possible to form microspots at a high accuracy and a high speed.

**[0070]** Moreover, a dispenser using the micropipette makes it possible to form microspots by efficiently dispensing hundreds to ten thousands of different samples at one time and thus, the productivity is remarkably improved.

**[0071]** The invention also consists in the methods of micropipetting herein described.

## Claims

1. A micropipette comprising:

   at least one substrate,
   an inlet port through which a sample is delivered from the outside, formed on said at least one substrate,
   a cavity into which the sample is poured and which is filled with the sample, and
   an injection port for expelling the sample formed on said at least one substrate,
   the substrate for forming the cavity being made of ceramics, at least one wall face of the substrate being provided with a piezoelectric/electrostrictive element, and the sample moving as a laminar flow in the cavity,

   wherein volumes of the cavity are changed by driving the piezoelectric/electrostrictive element and a certain amount of the sample in the cavity is expelled from the injection port.

2. The micropipette according to claim 1, wherein the cavity is previously filled with a displacement liquid, then a sample is poured into the cavity from the inlet port while laminar-flow-replacing the a displacement liquid with the sample, and thereafter a certain amount of the sample in the cavity is expelled from the injection port by driving the piezoelectric/electrostrictive element.

3. The micropipette according to claim 1, wherein the cavity is previously filled with a displacement liquid, a sample is poured into the cavity from the inlet port while driving the piezoelectric/electrostrictive element and thereafter, a certain amount of the sample in the cavity is expelled from the injection port by driving the piezoelectric/electrostrictive element.

4. The micropipette according to claim 2 or 3, wherein completion of laminar-flow replacement of the sample in the cavity is grasped by detecting the change

of fluid characteristics in the cavity.

5. A micropipette comprising:

at least one substrate,
an inlet port through which a sample is delivered from the outside, formed on said at least one substrate,
a cavity into which the sample is poured and which is filled with the sample, and
an injection port for expelling the sample formed on said at least one substrate,
the substrate for forming the cavity being made of ceramics, at least one wall face of the substrate being provided with a piezoelectric/electrostrictive element, volumes of the cavity being changed by driving the piezoelectric/electrostrictive element, and a certain amount of the sample in the cavity being expelled from the injection port;

wherein the cavity is previously filled with a displacement liquid, then a sample is poured into the cavity from the inlet port while replacing the displacement liquid with the sample, completion of replacement of the sample in the cavity is grasped by detecting the change of fluid characteristics in the cavity, and thereafter a certain amount of the sample in the cavity is expelled from the injection port by driving the piezoelectric/electrostrictive element.

6. The micropipette according to claim 4 or 5, wherein the change of fluid characteristics in the cavity is grasped by applying a voltage for exciting vibrations to the piezoelectric/electrostrictive element and detecting the change of electric constants due to the vibrations.

7. The micropipette according to any one of claims 1-6, wherein a plurality of the inlet ports, a plurality of the cavities, a plurality of the injection ports, and a plurality of the piezoelectric/electrostrictive elements are formed at one substrate.

8. The micropipette according to any one of claims 1-6, wherein a plurality of units in each of which the inlet port, the cavity, the injection port, and the piezoelectric/electrostrictive element are formed in/on the substrate one each is fixed to a fixing jig.

9. The micropipette according to any one of claims 1-6, wherein three types of portions of the combination of the cavity and the piezoelectric/electrostrictive element, the inlet port, and the injection port are separately formed on at least two types of substrates and joined each other.

10. The micropipette according to any one of claims 1-6 and 9, wherein at least the cavity and the piezoelectric/electrostrictive element are formed in/on the substrate, a unit in which at least one of the substrates is joined to a substrate with at least one of the inlet ports or the injection ports formed is formed, and at least one of the units is fixed and integrated.

11. The micropipette according to any one of claims 1-10, wherein the substrate is formed into a flat plate and the injection port is formed on a side face or a major surface of the substrate.

12. The micropipette according to any one of claims 1-10, wherein the substrate is formed into a flat plate, the injection port is formed on one of opposite major surfaces of the substrate, and the inlet port is formed on the other major surface of the substrate.

13. The micropipette according to any one of claims 1-12, wherein at least two of the inlet ports are connected to the cavity.

14. The micropipette according to any one of claims 1-13, wherein a substrate in/on which at least the cavity and the piezoelectric/electrostrictive element are formed is made of zirconia ceramics.

15. The micropipette according to any one of claims 1-14, wherein the substrate is made of zirconia ceramics.

16. The micropipette according to any one of claims 1-15, wherein the substrate is formed in accordance with a green-sheet laminating and sintering method.

17. The micropipette according to any one of claims 1-13, wherein a substrate in which at least one of the inlet ports and one of the injection ports are formed is made of a metal or a resin.

18. The micropipette according to any one of claims 1-17, wherein a piezoelectric/electrostrictive film of the piezoelectric/electrostrictive element is mainly made of a component consisting of lead zirconate, lead titanate, and lead magnesium niobate.

19. A dispenser using a plurality of micropipettes respectively formed so that inlet ports through which a sample is delivered from the outside, cavities to be filled with the sample, and injection ports for expelling the sample are formed on at least one substrate, a piezoelectric/electrostrictive element is provided for at least one wall surface of the substrate for forming the cavities, and the sample moves as a laminar flow in the cavity, wherein

the injection ports are vertically and horizon-

tally lined and arranged and different types of solution samples are injected from the injection ports.

**20.** The dispenser according to claim 19, wherein the cavities are previously filled with a displacement liquid, then different types of samples are poured into the cavities from the inlet ports while laminar-flow-replacing the displacement liquid with the sample, and thereafter the different types of samples in the cavities are expelled from the injection ports by driving the piezoelectric/electrostrictive element.

**21.** The dispenser according to claim 19, wherein the cavities are previously filled with a displacement liquid, then different types of samples are poured into the cavities from the inlet ports while laminar-flow-replacing the displacement liquid with the samples by driving the piezoelectric/electrostrictive element, and thereafter different types of the samples in the cavities are injected from the injection ports by driving the piezoelectric/electrostrictive element.

**22.** The dispenser according to claim 20 or 21, wherein completion of laminar-flow replacement of samples in the cavities is grasped by detecting the change of fluid characteristics in the cavities.

**23.** A dispenser using a plurality of micropipettes respectively formed so that inlet ports through which a sample is delivered from the outside, a cavity into which the sample is poured and which is be filled with the sample, and injection ports for expelling the sample are formed on at least one substrate, the substrate forming the cavity is made of ceramics, the substrate has a piezoelectric/electrostrictive element on at least one wall surface, the cavity is previously filled with a displacement solution, then the sample is poured into the cavity through the inlet ports while replacing the displacement solution with the sample, completion of sample replacement in the cavity is grasped by detecting the change of fluid characteristics in the cavity, and thereafter a volume of the cavity is changed by driving the piezoelectric/electrostrictive element and a certain amount of the sample in the cavity is expelled through the injection ports, wherein the injection ports are vertically and horizontally lined and arranged and different types of solution samples are expelled from the injection ports.

**24.** The dispenser according to claim 22 or 23, wherein fluid characteristics in the cavities are grasped by applying a voltage for exciting vibrations to the piezoelectric/electrostrictive element and detecting the change of electric constants due to the vibrations.

**25.** The dispenser according to any one of claims 19-24, wherein a mechanism is included in which cartridges separately filled with different types of solution samples are set to the inlet ports one to one to deliver different solution samples from the inlet ports.

**26.** The dispenser according to any one of claims 19-25, wherein a mechanism is included in which a cartridge filled with a water-soluble solvent or organic solvent is set to each of the inlet ports to clean the space expanding from the inlet ports up to the injection ports formed in the substrate.

**27.** The dispenser according to any one of claims 19-26, wherein a different-directional-flying-droplet shielding plate of a thin plate having a hole coaxial with the central axis of each of the injection ports is provided on the outside of each of the injection ports.

Fig. 1

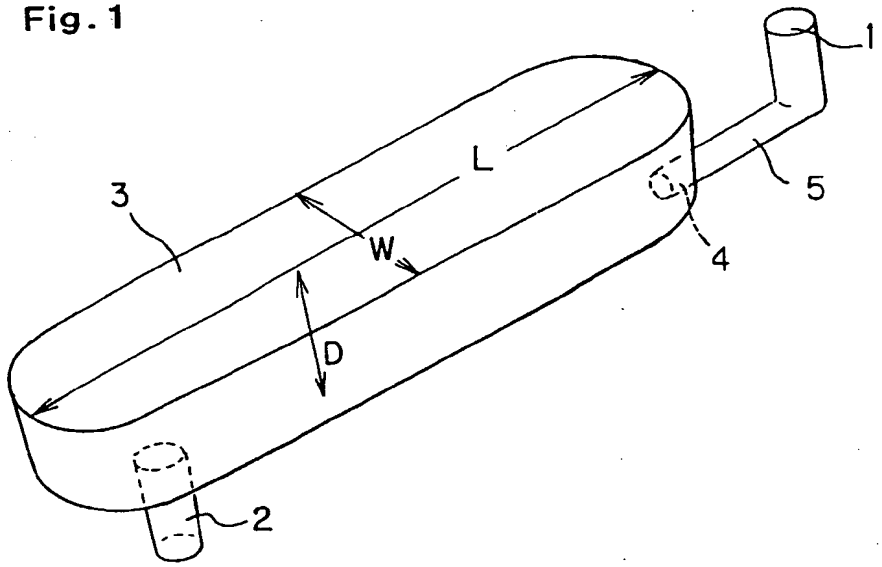



Fig. 2

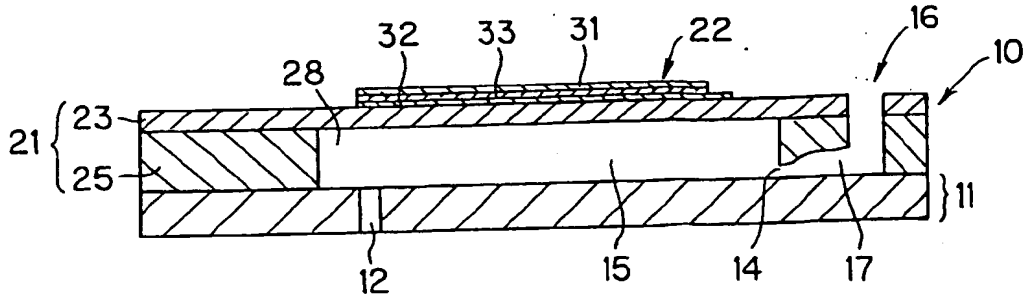


Fig.3(a)

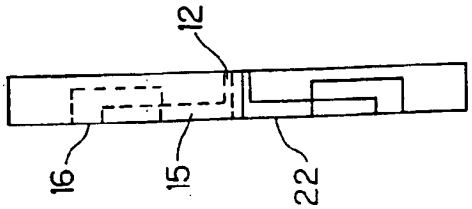



Fig.3(b)

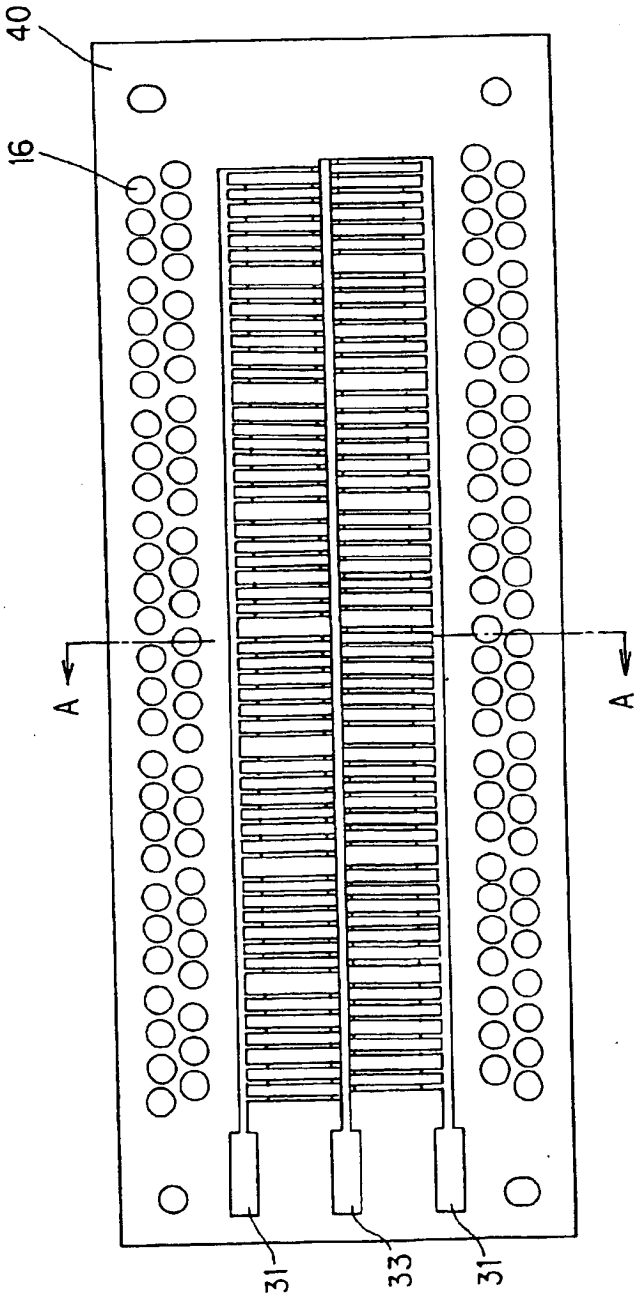


Fig.4(a)

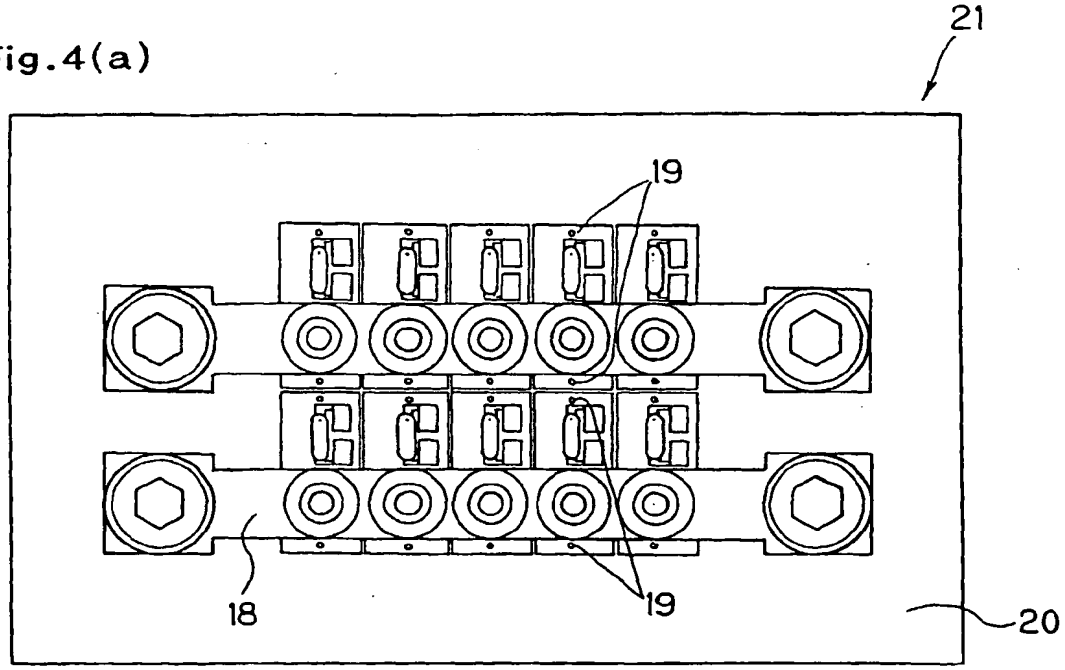



Fig.4(b)

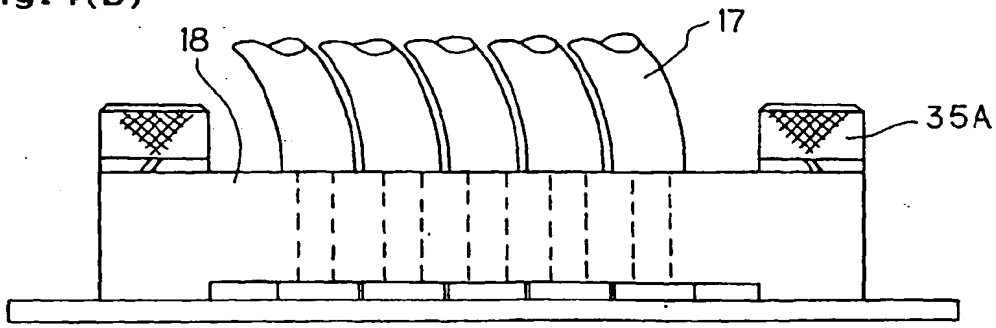



Fig.4(c) Fig.4(d)

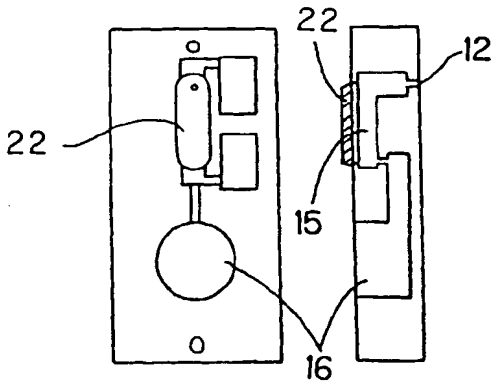


Fig.5(a)

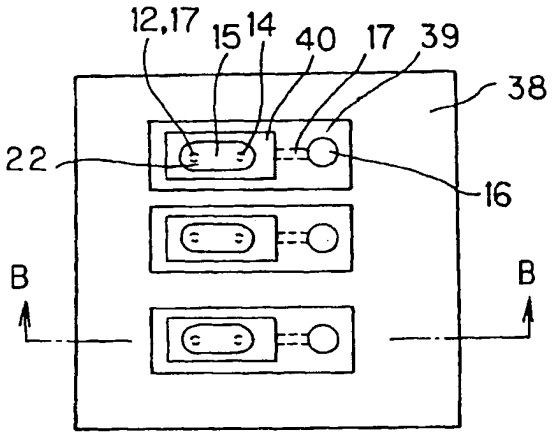



Fig.5(b)

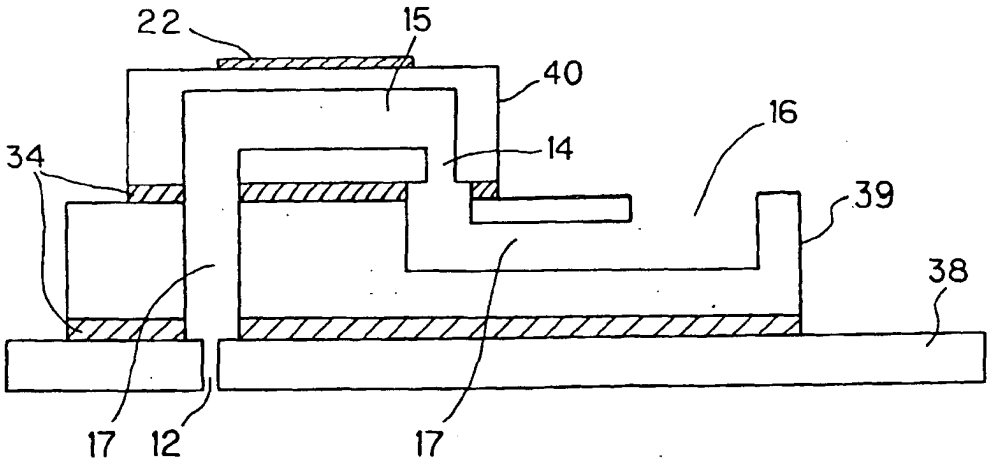


Fig.6(a)

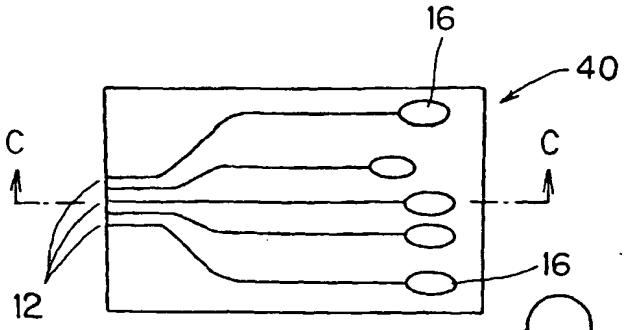



Fig.6(b)

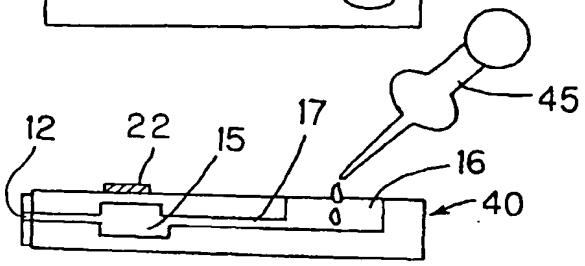



Fig.7(a)

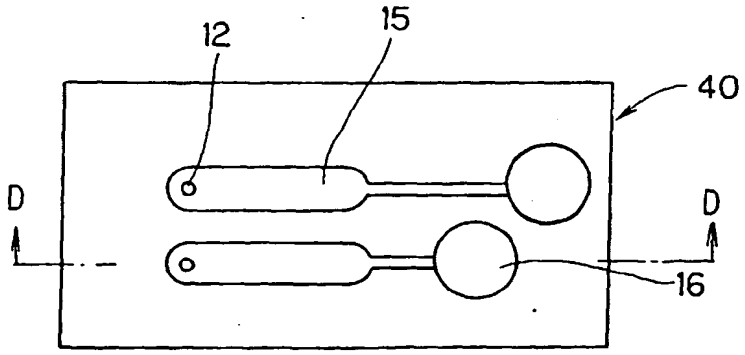



Fig.7(b)

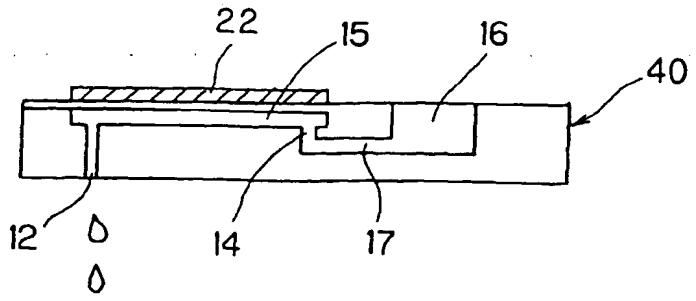


Fig.8(a)

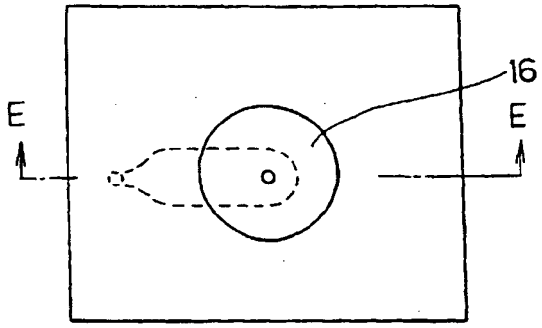



Fig.8(b)

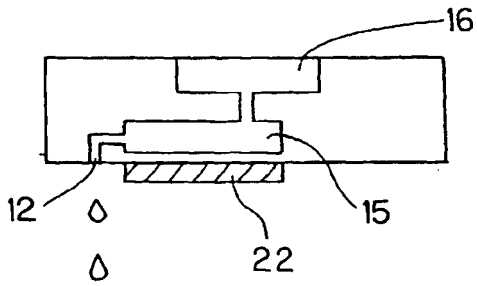



Fig.9(a)

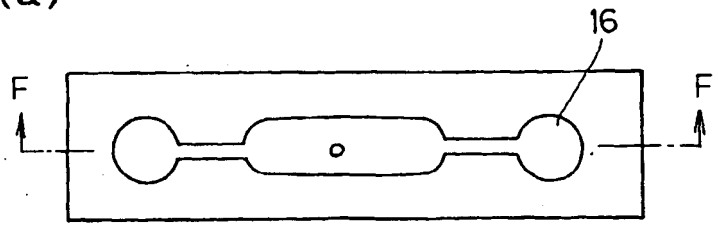



Fig.9(b)

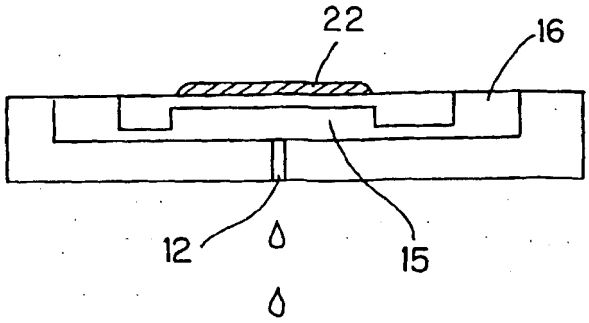


Fig. 10

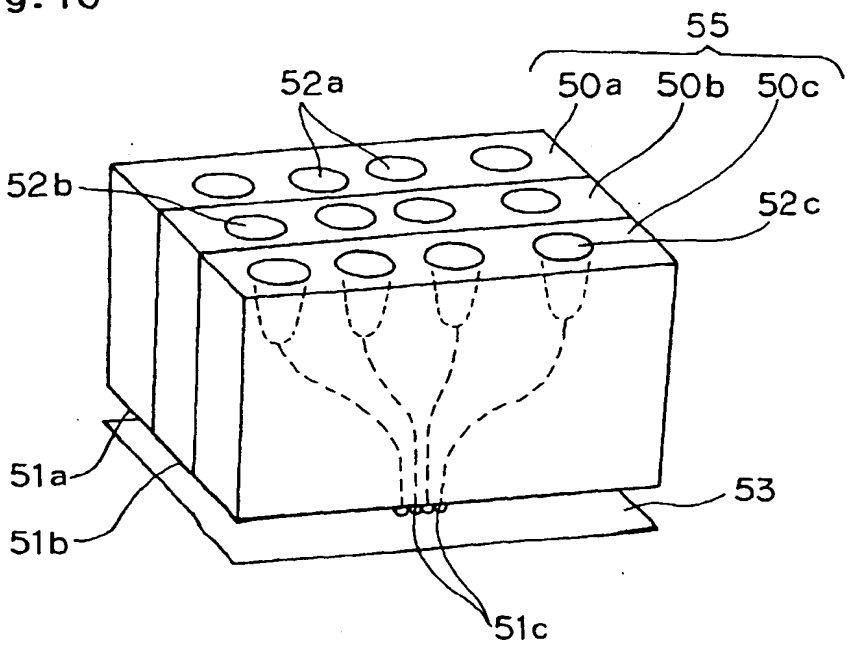



Fig. 11(a)

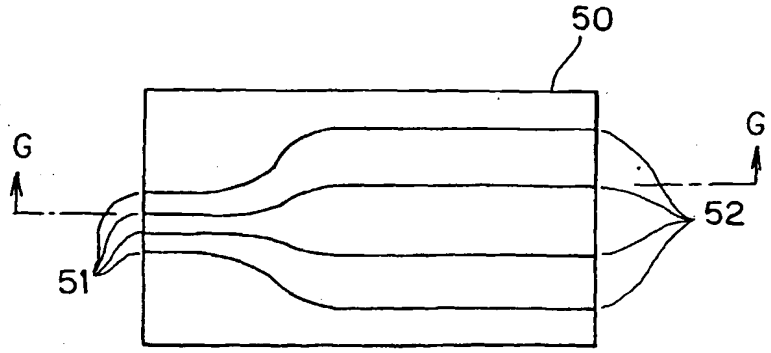



Fig. 11(b)

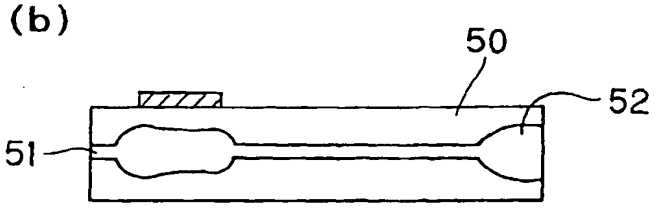
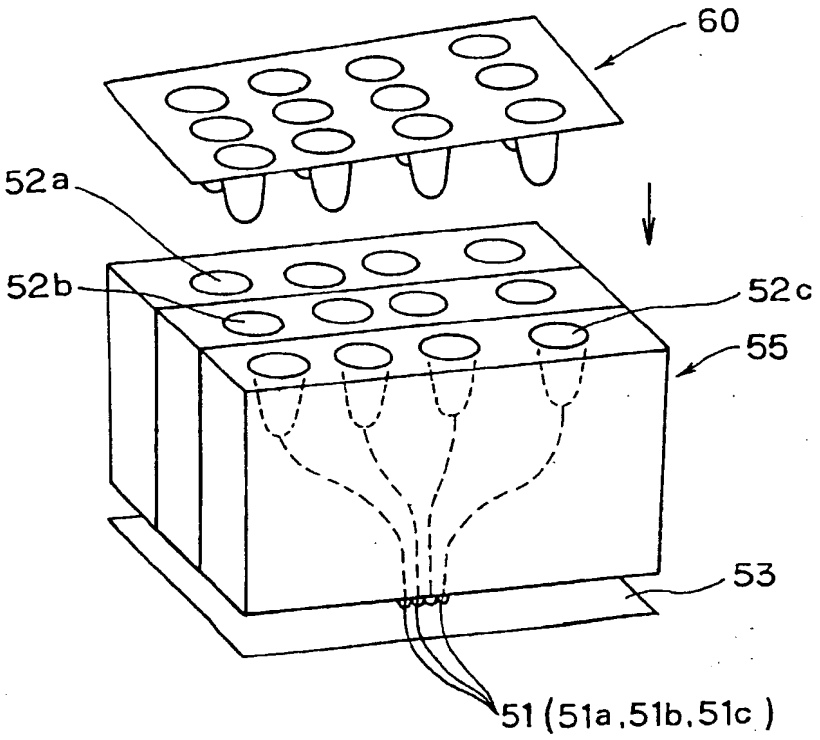


Fig. 12



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 093 855 A3

# (12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
**18.06.2003 Bulletin 2003/25**

(43) Date of publication A2:
**25.04.2001 Bulletin 2001/17**

(21) Application number: **00309286.3**

(22) Date of filing: **20.10.2000**

(51) Int Cl.[7]: **B01L 3/02**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE**
Designated Extension States:
**AL LT LV MK RO SI**

(30) Priority: **22.10.1999 JP 30162799**

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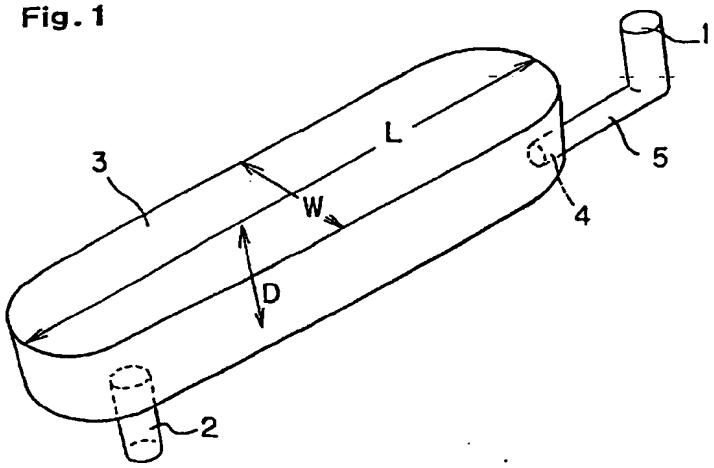
## (54) Micropipette and dispenser

(57) A micropipette includes: at least one substrate, an inlet port through which a sample is delivered from the outside, a cavity to be poured and filled with the sample, and an injection port for expelling the sample are formed on the at least one substrate. The substrate for forming the cavity is made of ceramics, a piezoelectric/electro-strictive element is provided for at least one wall surface of the substrate, and the sample moves as a laminar flow in the cavity. Volumes of the cavity are changed by driving the piezoelectric/electrostrictive element to expell a certain amount of the sample in the cavity from the injection port. According to the micropipette, it is possible to form microspots at a high accuracy and a high speed. According to a dispenser using the micropipette, it is possible to efficiently dispense hundreds to ten thousands of different samples at one time and form microspots. Therefore, the productivity is remarkably improved.

Fig. 1




EP 1 093 855 A3

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number
EP 00 30 9286

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.7) |
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